

Natural *Leptospira pomona* Infection in the Pampas Cavy

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AN OUTBREAK of leptospirosis in Hereford cattle on a large ranch in the central part of the Province of Corrientes, Argentina, was characterized by premature calving, 2 to 4 weeks short of full-term pregnancy, with the fetus usually dead on delivery. Calves up to 6 months old also suffered considerable mortality. Serodiagnosis, by the microscopic agglutination technique, revealed positive titers for *Leptospira pomona* in the cattle, and the diagnosis was further confirmed by the isolation of that serotype from a 5-month-old calf.

The average number of cattle at the ranch is about 19,000. The only entries to the herd from outside sources are some 200 breeding bulls which are added each year.

Information given by the owners of the ranch indicated that the disease probably had been present for at least 3 years before it was identified, and it had been diagnosed as brucellosis, anaplasmosis, or piroplasmosis.

With leptospirosis in cattle confirmed, we decided to investigate various epizootiological aspects of the problem, including the possible role of wild mammals in the perpetuation and dissemination of the disease. Several available species of wild mammals were examined, among them the native cavy, *Cavia pamparum* Thomas, which was especially common in the district. The results obtained with respect to this indigenous rodent are presented here.

The ranch which provided most of the animals for this study contains some 34,500 hectares (1 hectare = 2.4715 acres) of rolling grassland, divided into 65 paddocks (fields) of varying sizes. The land is drained by mean-

dering streams, some of which are intermittent. One stream crosses the ranch, and three other streams border about one-half of its periphery. All of these streams flow throughout the year.

Methods

The cavies were collected on three occasions in 1961: April 25-26, August 20-24, and December 4-6. On the first trip all animals were caught in a single large paddock, about 300 hectares, which was selected because livestock infection appeared to be centered there. This field, L.E., is crossed by a stream which flows constantly through a sector partially covered with bunchgrass. The first lot of cavies was captured in this sector in an area of about 4 hectares.

On the second trip cavies were again captured in this sector, as well as in seven other paddocks of the same ranch and in one paddock, CPCY, of an adjoining ranch. The third lot was collected from only three of the paddocks previously sampled, including L.E.

After attempts to capture the animals with several models of live traps failed, we resorted to capture by hand. With the help of several ranch employees, we caught a total of 282 cavies. Each animal was given an accession number, exsanguinated, and autopsied. Weight, total length, and sex were recorded for each.

The surface of the bladder was seared with a

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hot spatula, and a urine sample removed with a sterile syringe. A tube containing 5 ml. of Fletcher's medium (enriched with 10 percent rabbit serum) was inoculated with 1 drop of urine. The dilution technique of Menges and associates (1) was used to make serial tenfold dilutions of urine with buffered physiological saline ($\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1,000}$, and $\frac{1}{10,000}$). A single drop of each dilution was inoculated into a tube with Fletcher's medium. Urine samples were cultured from all cavies, except a few from which samples were unobtainable.

Kidney tissue was cultured in the manner described by Wolff (2). The surface of the kidney was seared, a small plug of renal cortex was removed with a Pasteur pipette, and the material was inoculated into Fletcher's medium. One tube of medium was inoculated from each kidney, two tubes per animal.

Collection of blood samples and inoculation of culture media were performed on some of the cavies in the field within 24 hours after capture. Although other cavies had to be transported alive by air courier to the Pan American Zoonoses Center laboratories for processing, the elapsed time between capture and autopsy never exceeded 72 hours. Exposure to other animals or possible sources of infection was minimal.

Tubes of inoculated medium were incubated at 28° C. and examined weekly for 6 weeks. When growth of leptospire was detected, successive transfers were made in Stuart's liquid medium (with 10 percent rabbit serum) until

Monthly rainfall registered at ranch from which wild cavies were captured during 1961, Corrientes Province, Argentina

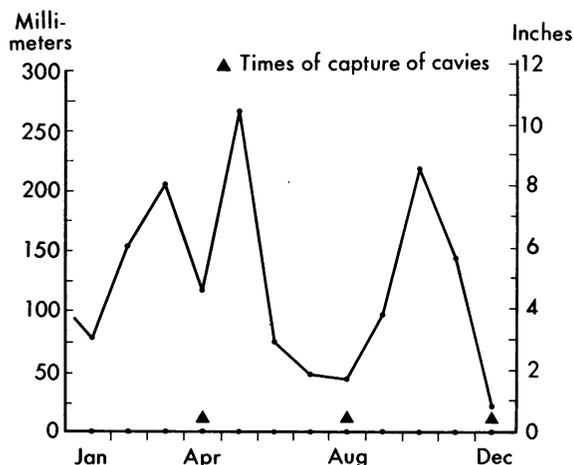


Table 1. Results of tests for *Leptospira pomona* in wild cavies captured in cattle paddocks, Corrientes Province, Argentina, 1961

Month of capture and paddock designation ¹	Number of cavies	Positive by serology		Positive by culture	
		Number	Per cent	Number	Per cent
<i>April</i>					
L.E.-----	57	6	10	3	5
<i>August</i>					
L.E.-----	26	7	27	3	11
No. 4-----	28	4	14	1	3
No. 6-----	30	1	3	0	-----
No. 10-----	4	0	-----	0	-----
No. 25-----	14	0	-----	0	-----
R.Y.-----	12	1	8	0	-----
La Esp-----	7	0	-----	0	-----
S.C.-----	1	0	-----	0	-----
CPCY-----	13	0	-----	0	-----
<i>December</i>					
L.E.-----	21	5	24	4	19
No. 4-----	29	1	3	0	-----
No. 6-----	40	0	-----	0	-----
Total-----	282	25	9	11	4

¹ All paddocks are on one ranch except CPCY which is on adjoining ranch.

a growth satisfactory for use as antigen was obtained. Leptospiral isolates were screened with a battery of rabbit antisera of the following serotypes: *L. icterohaemorrhagiae*, *L. canicola*, *L. pomona*, *L. autumnalis*, *L. ballum*, *L. bataviae*, *L. pyrogenes*, *L. australis*, *L. sejroe*, *L. grippotyphosa*, *L. hebdomadis*, and *L. hyos*. Specific antiserum was prepared for each isolate, using a procedure of the Walter Reed Army Institute of Research (3), and cross agglutination absorption tests were performed for each isolate, according to the technique described by Galton and associates (4).

The serum of each animal was subjected to a screening test at a final dilution of 1:50, using the microscopic agglutination test with live antigens (agglutination-lysis test) for 12 serotypes. Serums that reacted to the screening test were tested further in serial fourfold dilutions with the corresponding antigens to determine the end-point titer. A reaction of at least 50 percent agglutination was considered positive. The procedure used for testing and

for interpreting the results was that employed at the Public Health Service Communicable Disease Center, Atlanta, Ga. (4).

The pH of soil samples from three paddocks was determined with a Beckman pocket pH meter. Two samples of soil from each of these paddocks, L.E., No. 4, and No. 6, were examined.

Results

During the three trips, a total of 282 cavies were captured, autopsied, and examined serologically as well as culturally for leptospirosis.

Agglutination tests. Twenty-five (9 percent) of the 282 cavies had an agglutination titer for *L. pomona* (table 1), ranging from 1:50 to 1:12,800 (table 2). Serologically

positive cavies were found on four of the eight paddocks of the ranch. The 13 cavies captured at an adjoining ranch were all negative.

Of special interest are the results obtained in paddock L.E., where most of the bovine infections occurred and where cavies were captured at intervals of 4 months on the three occasions. Comparison of the results of the three samplings shows a significant increase of reactors in this paddock, from 10 percent in April to 27 percent in August and 24 percent in December (table 1). A total of 104 cavies were captured in this paddock on the three occasions, and 18 (17.3 percent) showed serologic evidence of *L. pomona* infection.

Cross-reactions for other serotypes (*L. grippotyphosa*, *L. icterohaemorrhagiae*, and *L.*

Table 2. Positive results for leptospirae, by serologic and cultural methods, in 25 of 282 wild cavies captured in cattle paddocks, Corrientes Province, Argentina, 1961

Month of capture and paddock designation	Sex	Weight (grams)	Agglutination-lysis titers by serotype				Culture		Serotype isolated
			<i>L. pomona</i>	<i>L. grippotyphosa</i>	<i>L. icterohaemorrhagiae</i>	<i>L. autumnalis</i>	Kidney	Urine	
<i>April</i>									
L.E.-----	M	280	1:50	—	—	—	+	—	<i>L. pomona</i>
L.E.-----	M	700	1:50	—	—	—	—	—	
L.E.-----	M	795	1:50	—	—	—	—	—	
L.E.-----	M	660	1:800	1:200	1:50	1:50	+	+	<i>L. pomona</i>
L.E.-----	M	650	1:200	—	—	—	+	(¹)	
L.E.-----	M	590	1:800	—	—	—	—	—	<i>L. pomona</i>
<i>August</i>									
L.E.-----	M	610	1:12,800	1:200	—	1:200	+	—	<i>L. pomona</i>
L.E.-----	M	590	1:3,200	1:50	—	—	+	—	
No. 6-----	F	480	1:800	—	—	—	—	—	<i>L. pomona</i>
L.E.-----	F	600	1:200	—	—	—	—	—	
L.E.-----	M	650	1:50	—	—	—	—	—	<i>L. pomona</i>
L.E.-----	M	650	1:50	—	—	—	—	+	
L.E.-----	F	520	1:200	—	—	—	—	—	
L.E.-----	M	620	1:200	1:200	—	—	—	—	<i>L. pomona</i>
R.Y.-----	M	650	1:50	—	—	—	—	—	
No. 4-----	M	650	1:50	—	—	—	—	+	<i>L. pomona</i>
No. 4-----	M	700	1:50	—	—	—	—	—	
No. 4-----	F	610	1:800	—	—	—	—	—	<i>L. pomona</i>
No. 4-----	M	595	1:50	—	—	—	—	—	
<i>December</i>									
L.E.-----	M	550	1:50	—	—	—	+	+	<i>L. pomona</i>
L.E.-----	M	480	1:3,200	—	—	—	+	(¹)	
L.E.-----	M	700	1:50	—	—	—	—	—	<i>L. pomona</i>
L.E.-----	M	400	1:50	—	—	—	—	+	
L.E.-----	M	480	1:3,200	—	—	—	—	+	<i>L. pomona</i>
No. 4-----	M	630	1:50	—	—	—	—	—	

¹ Urine not cultured.

autumnalis) were observed in only four animals, and *L. pomona* was isolated from two of these.

Data on sex and weight were obtained for 265 animals (table 3). Of the 135 males examined by the microscopic agglutination test, 21 (15.5 percent) showed titers for *L. pomona*, whereas of 130 females tested only 4 (3 percent) reacted to a titer 1:50 or higher. Most of the animals captured weighed more than 400 gm. Thirty of the 265 cavies weighed less than 400 gm., and only one of the lower-weight animals reacted serologically.

Cultures. A total of 13 leptospiral isolates were obtained from the kidneys or urine, or both, of 11 (4 percent) of the 282 cavies (tables 1 and 2). Urine was not examined from 2 of the 11 culturally positive animals. Of the remaining nine cavies from which both kidney tissue and urine were cultured, in three instances isolates were obtained from kidney tissue only, in four from urine only, and in two from both sources. It is noteworthy that urinary shedding was confirmed in six of nine animals.

All the culturally positive cavies, with the exception of one, came from paddock L.E. Of the 104 cavies from this paddock, 10 (9.6 percent) were culturally positive.

A breakdown of results by sex (table 3) shows that of 135 males examined 11 (8.1 percent) were culturally positive, and that no isolates were obtained from a comparable group of 130

females. Ten of the 11 positive males weighed more than 400 gm.

Serum from the 11 culturally positive cavies reacted to the microscopic agglutination test with *L. pomona* antigen, although in six the titer was only 1:50.

Environmental data. The monthly rainfall registered at the ranch during 1961 is shown in the chart. Two soil samples from paddock No. 4 gave pH readings of 5.4 and 5.85, and two samples from paddock No. 6 gave 5.65 and 5.8. Two samples from paddock L.E. gave 6.85 and 8.2, the alkaline reading corresponding to the sector which abounded in cavies and from which the majority of the leptospiral positive animals were captured.

Discussion

The pampas cavy is found throughout north-eastern Argentina, including the Provinces of Corrientes and Entre Rios, in a large part of Santa Fe Province, and throughout the Province of Buenos Aires except perhaps the extreme southern part (5). The animal is also abundant in Uruguay, having been described as the most common rodent in that country (6). The distribution includes practically all of the "Pampasico" and the southern half of the "Subtropical" Zoogeographical Districts of South America, as described by Cabrera and Yepes (7). This extensive area of humid grasslands is outstanding for its cattle production.

Table 3. Sex and weight distribution of 265 cavies positive for *Leptospira pomona*, Corrientes Province, Argentina, 1961

Weight group (grams)	Males			Females			Both sexes		
	Total	Number serologically positive	Number culturally positive	Total	Number serologically positive	Number culturally positive	Total	Number culturally positive	Number positive for <i>L. pomona</i>
Less than 100.....	0	0	0	1	0	0	1	0	0
100-199.....	3	0	0	4	0	0	7	0	0
200-299.....	4	1	1	5	0	0	9	1	1
300-399.....	3	0	0	10	0	0	13	0	0
400-499.....	22	3	3	42	1	0	64	4	3
500-599.....	37	4	2	40	1	0	77	5	2
600-699.....	49	9	5	24	2	0	73	11	5
700-799.....	11	4	0	2	0	0	13	4	0
800 or more.....	6	0	0	2	0	0	8	0	0
Total.....	135	21	11	130	4	0	265	25	11

Rodents have been long recognized as important reservoirs in nature for most serotypes of leptospire. However, with some exceptions, such as in Denmark (8) or eastern Slovakia (9) where the field mouse, *Apodemus agrarius*, is considered the principal source of *L. pomona* infection for domestic livestock or human beings, and in north Queensland where *Rattus assimilis* is the principal natural reservoir (10,11), rodents are not generally considered important in the maintenance of that serotype in nature. In the Americas, the only hitherto recorded instance of the isolation of *L. pomona* from rodents was from a woodchuck, *Marmota monax monax*, in the State of Pennsylvania (12).

The results obtained in the investigation reported here provide strong evidence of relationship between leptospirosis in cattle and the pampas cavy in the milieu studied. The fact that almost 10 percent of the cavy, captured on three occasions at 4-month intervals, showed evidence of past or present infection suggests that the cavy population was involved in an active wave of infection due to *L. pomona*.

The first lot of cavy was captured in a limited sector of paddock L.E. This paddock was selected because the incidence of bovine leptospirosis was highest in that area. The sector within the paddock was chosen because it offered the habitat best suited to cavy, as shown by their relative abundance. Based on the positive serologic and cultural results obtained from the first sampling of animals from that sector, new samples were studied on each of the two succeeding visits. The results indicate that the infection was maintained in the sector throughout the study, and that, in fact, there was a significant increase in the infection rate. The incidence of urinary shedders among the cavy from this paddock was high.

There is little doubt that the high concentration of the cavy and the high incidence rate of leptospirosis among them may have contributed to the distribution and perpetuation of infection. In this respect, it is noteworthy that *L. pomona* seemed to be well adapted to the cavy as no clinical symptoms of disease were noted in the captured animals and no lesions attributable to leptospirosis were observed on necropsy. The pampas cavy is very often found

along natural water reservoirs and streams where domestic animals obtain their drinking water. These streams may serve as a vehicle of the infective agent in interspecies transmission.

Undoubtedly the number of positive cultures from renal tissue could have been increased, if different concentrations of kidney suspensions had been employed for inoculations according to the technique described by Galton, Menges, and Shotts (4,13). This method, however, was not feasible under the working conditions of this study.

The isolation of leptospire, in three instances from kidney tissue only and in four instances from urine only, supports the recommendation of Menges and Galton (13) that both kidney tissue and urine should be cultured at necropsy.

As might be expected, there was a certain degree of serologic cross-reaction with antigens of other serotypes. Interestingly, *L. pomona* was isolated from five cavy with titers of only 1:50 for that serotype. These findings emphasize the inadequacy of serologic studies alone to determine the prevalence of leptospiral infection.

A striking sex difference was found in prevalence, as all *L. pomona* isolates were from males and only 4 of the 25 seropositive cavy were females. No explanation can be given at present for this phenomenon. In a general review of the literature, Rosenberg (14) noted a similar situation in dogs. He estimated that the incidence of leptospirosis among male dogs is 3 to 5 times higher than among bitches.

Analysis of the data on body weights of the positive cavy in relation to the total number studied shows that the rate of infection was markedly higher in the heavier animals. Assuming that weight is an indicator of age, the infection rate is much higher in old than in young cavy. Gordon-Smith and associates reported a general tendency for the incidence of leptospiral infection to rise with age in several Malayan rat species (15).

During the course of the present investigation, workers in São Paulo, Brazil, reported the isolation of *L. icterohaemorrhagiae* from another species of cavy (*Cavia aperea azarae*) captured in that city (16). Rodents of the several species of the subfamily *Caviinae* (cavy)

are widely distributed in South America, and systematic studies should be carried out to determine the extent of their involvement in the natural history of leptospirosis.

Summary and Conclusions

The natural occurrence of *Leptospira pomona* infection in the pampas cavy, *Cavia pamparum*, was investigated in connection with an outbreak of leptospirosis in cattle at a large ranch in the central part of Corrientes Province, Argentina. Of 282 cavies, captured and examined on three occasions during 1 year, 25 were serologically positive for *L. pomona*, and the organism was isolated from urine or kidney tissue of 11. None of the cavies showed clinical signs of disease.

To our knowledge, this is the first reported isolation of *L. pomona* from wild cavies, and the first evidence that these animals may play a role in the epizootiology of bovine leptospirosis. Because the pampas cavy is prevalent throughout the most important cattle-producing areas of Argentina and Uruguay, further studies are indicated to define its true role as a reservoir of leptospirosis transmissible to domestic livestock and to the rural human population.

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Syphilis Continues to Increase

Reported cases of primary and secondary syphilis totaled 21,143 in 1962. This is the highest annual total since 1950 and represents a continuation of the increase which began in 1956, when 6,392 cases were reported. The most marked rise in the past 6 years occurred in 1960, when reported infectious syphilis cases increased 65 percent over the preceding year. The Public Health Service is currently intensifying a program based on epidemiologic investigation and education with the goal of eradicating syphilis in the United States.